CAMILA SCHULTZ MARCOLLA

CHROMIUM YEAST IMPROVES EFFICIENCY AND CARCASS QUALITY OF PIGS FED RACTOPAMINE

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

VIÇOSA
MINAS GERAIS -BRAZIL
2017

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
M321c
2017

Marcolla, Camila Schultz, 1989-
Chromium yeast improves efficiency and carcass quality of pigs fed ractopamine / Camila Schultz Marcolla. – Viçosa, MG, 2017.
ix, 25f. : il. ; 29 cm.

Orientador: Alysson Saraiva.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Suíno - Alimentação e rações. 2. Nutrição animal. 3. Cromo na nutrição animal. 4. Carne - Qualidade. I. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-graduação em Zootecnia. II. Título.

CDD 22 ed. 636.085

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APPROVED: March 28, 2017.

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(Advisor)

"...a mind needs books as a sword needs a whetstone if it is to keep its edge."

(George R. R. Martin)

ACKNOWLEDGEMENTS

The present work was supported by CNPq, the National Council for Scientific and Technological Development.

I would like to thank my advisor, professor Alysson Saraiva, whose guidance and support were fundamental to this research. Also, I would like to thank the members of the examination committee for their contribution to this work.

I would like to thank the help provided by the staff and the undergraduate students working on the swine research facility of UFV, for their valuable and constant help, which were fundamental throughout the experimental period. I also would like to thank Valdir Ribeiro, for the help with the statistical analysis, to dear professor Melissa Hannas, for her support, and to the staff and students of Meat Science and Animal Nutrition laboratories.

I am very grateful to my classmates, Débora Muratori, Lívia Barbosa and Soraia Ferreira, who helped me along the way. I wish to thank Soraia and Débora for helping me since my pigs arrived until the end of laboratory work. Above that, I thank they for being such good friends, helping me beyond academic level and improving my personal life in the past two years (and in the years to come).

Finally, and more important, I would like to thank my family for their support and encouragement throughout the course of this study, as it has been throughout all my life. To my parents, Glória and Alexandre, for their unconditional love and their willingness to make my life as easy as possible, embracing, with enthusiasm, every decision I make. To my sister, Débora, for all the love, patience, and for Ana Laura. To Levi, for his support, companionship, patience, and for having lent me his family which now is mine as well. I am deeply thankful to Maria, Vicente, Lorena, Davi, Marina, and Fernando for the way they received me in Viçosa.

TABLE OF ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
BF	back fat depth
BW	body weight
CLA	conjugated linoleic acid
d	day
G:F	growth-to-feed ratio
LM	Longissimus muscle
wk	week

ABSTRACT

MARCOLLA, Camila Schultz, M.Sc., Universidade Federal de Viçosa, March, 2017. **Chromium yeast improves efficiency and carcass quality of pigs fed ractopamine.** Advisor: Alysson Saraiva.

This study aimed to evaluate the effects of dietary chromium, CLA, and ractopamine on performance and pork quality of finishing pigs slaughtered at 115 kg BW. Ninety-six crossbred barrows (initial BW= 70.21 ± 1.98 kg) were randomly assigned to 1 of 6 dietary treatments. A diet formulated according to the nutritional requirements of 70- to 100-kg barrows of high lean genotype gaining 1.13 kg/day was used as the control (CON). The other five diets were based on the CON and supplemented as follows: 400 ppb Cr yeast (CrY); 0.5% CLA (CLA); 400 ppb CrY and 0.5% CLA (CrY+CLA); 20 ppm RAC (RAC); 400 ppb CrY and 20 ppm RAC (CrY+RAC). Lysine levels on diets containing RAC were raised by 20% compared to CON. Pigs fed RAC and CrY+RAC were fed CON for the first 17 d, and then the respective diets for the last 28 d on trial. There were 8 replicates per treatment (48 pens; 2 pigs per pen). Means were compared using Tukey test, excluding the CON. Dunnett test was used to compare the means of each diet containing additives to the CON. Initial BW was used as covariate. Pigs fed RAC and CrY+RAC had the highest ($P < 0.01$) final BW and ADG. Pigs fed CrY+RAC had higher ($P < 0.01$) G:F than pigs fed diets containing additives, except for the RAC. Pigs fed CrY+RAC and RAC had similar G:F, both higher ($P < 0.01$) than pigs fed CON. Feed intake was similar ($P = 0.89$) for all diets. Pigs fed CrY+RAC had higher LM area ($P < 0.05$) and carcass yield ($P < 0.01$) than pigs fed CON, CrY, CLA, and CrY+CLA. Loin muscle area and carcass yield of pigs fed RAC were not statistically different from pigs fed the others diets tested. Back fat depth was lower ($P = 0.22$) in pigs fed CLA, RAC and CrY+RAC compared to pigs fed CON. The additives did not affect pork pH ($P > 0.05$), temperature ($P > 0.05$), water losses ($P = 0.87$), and shear force ($P = 0.70$). Lower L^* values were found on pork from pigs fed CrY+CLA compared to pigs fed CON ($P < 0.05$), and RAC ($P < 0.01$). Pigs fed the RAC had lower ($P < 0.05$) a^* values compared to pigs fed CON. Pork b^* values were similar ($P = 0.20$) for all diets. There were no significant differences ($P = 0.89$) on intramuscular fat content. Serum urea nitrogen (SUN) concentration was lower ($P = 0.03$) in pigs fed CrY+RAC than in pigs fed CON and RAC. In summary, when combined, CrY and RAC increase LM area and carcass yield and reduce SUN, suggesting a synergism

that might be related to the ability of chromium to improve nutrient utilization in RAC-fed pigs. Additionally, CrY, CLA and RAC have no major effects on pork quality.

RESUMO

MARCOLLA, Camila Schultz, M.Sc., Universidade Federal de Viçosa, março de 2017. **Cromo levedura melhora a eficiência e a qualidade da carcaça de suínos alimentados com ração contendo ractopamina.** Orientador: Alysson Saraiva.

Este trabalho foi conduzido com o objetivo de avaliar os efeitos da adição de cromo, ácido linoleico conjugado, e ractopamina no desempenho, características de carcaça e qualidade de carne de suínos abatidos aos 115 kg. Foram utilizados 96 suínos machos castrados com peso inicial de 70,21 ± 1,98 kg. Os animais foram distribuídos ao acaso em seis tratamentos. Foram utilizadas oito repetições por tratamento e dois animais por unidade experimental (48 baias, 2 suínos por baia). Os tratamentos consistiram de uma dieta controle (CON) formulada para atender as exigências nutricionais de suínos machos castrados de alto potencial genético. As demais dietas foram formuladas utilizando a dieta CON como base, à qual foram adicionados os aditivos a serem testados, da seguinte maneira: 400 ppb de cromo levedura (CrL); 0,5% de CLA (CLA); 400 ppb CrL e 0,5% CLA (CrL+CLA); 20 ppm de ractopamina (RAC); 400 ppb cromo levedura e 20 ppm de ractopamina (CrL+RAC). Os níveis de lisina nas dietas contendo ractopamina foram aumentados em 20% em relação à dieta CON. Os suínos alimentados com RAC e CrL+RAC receberam a dieta CON durante os primeiros 17 dias de experimento, e passaram a consumir as respectivas dietas nos últimos 28 dias de experimento. As médias entre os tratamentos contendo aditivos foram comparadas usando o teste Tukey. A média de cada tratamento contendo aditivos foi comparada à média da dieta CON utilizando o teste Dunnett. O peso médio inicial foi usado como covariável. Os suínos alimentados com RAC e CrL+RAC apresentaram maior ($P < 0,01$) peso final e ganho de peso médio diário do que os suínos dos demais tratamentos. A eficiência alimentar foi maior ($P < 0,01$) nos suínos recebendo CrL+RAC em comparação com os suínos recebendo dieta contendo aditivos, com exceção dos animais alimentados com RAC, que tiveram eficiência alimentar similar aos alimentados com CrL+RAC. Suínos alimentados com RAC e CrL+RAC apresentaram eficiência alimentar semelhantes, ambas superiores ($P < 0,01$) à eficiência alimentar dos animais alimentados com dieta CON. O consumo de ração foi semelhante ($P = 0,89$) para todas as dietas. Suínos alimentados com CrL+RAC apresentaram maior área de olho de lombo (AOL) ($P < 0,01$) e rendimento de carcaça ($P < 0,05$) do que os animais

alimentados com as dietas CON, CrL, CLA e CrL+CLA. Os resultados de AOL e rendimento de carcaça de suínos alimentados com RAC foram similares aos resultados obtidos nos animais alimentados com as demais dietas. Os animais alimentados com CLA, RAC e CrL+RAC apresentaram menor (P = 0,22) espessura de toucinho quando comparados aos animais recebendo dieta CON. Os aditivos não influenciaram o pH (P > 0,05), a temperatura (P > 0,05), as perdas de água (P = 0,87) e a força de cisalhamento (P = 0,70) da carne. Menores valores de *L** foram encontrados na carne de suínos alimentados com CrL+CLA em comparação aos suínos alimentados com CON (P < 0,05) e RAC (P < 0,01). Suínos alimentados com RAC apresentaram menores (P < 0,05) valores de *a** em comparação aos suínos alimentados com CON. Os valores de *b** foram semelhantes (P = 0,20) para todas as dietas. Não houve diferença significativa (P = 0,89) na concentração de gordura intramuscular. Não foram observadas diferenças nas concentrações séricas de glicose (P = 0,32), colesterol (P = 0,67) e triglicerídeos (P = 0,46). A concentração sérica de ureia foi menor (P = 0,03) nos suínos alimentados com CrL+RAC em comparação aos suínos alimentados com CON e RAC. A suplementação combinada de cromo e ractopamina aumenta a AOL e o rendimento de carcaça de suínos em terminação, sugerindo a existência de um sinergismo que pode estar relacionado a capacidade de cromo de aumentar a utilização de nutrientes em suínos suplementados com ractopamina. Os aditivos suplementados não apresentam efeitos importantes sobre a qualidade de carne.

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Chromium yeast improves feed efficiency and carcass quality of finishing pigs fed ractopamine

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ABSTRACT

This study aimed to evaluate the effects of dietary chromium, CLA, and ractopamine on performance, carcass traits, and pork quality of finishing pigs slaughtered at 115 kg BW. Ninety-six crossbred barrows (initial BW= 70.21 ± 1.98 kg) were randomly assigned to 1 of 6 dietary treatments. There were 8 replicates per treatment (48 pens; 2 pigs per pen). A diet formulated according to the nutritional requirements was used as the control (CON). The other 5 diets were based on the CON and supplemented as follows: 400 ppb Cr yeast (CrY); 0.5% CLA (CLA); 400 ppb CrY and 0.5% CLA (CrY+CLA); 20 ppm RAC (RAC); 400 ppb CrY and 20 ppm RAC (CrY+RAC). Lysine levels on diets containing ractopamine were raised by 20% compared to CON to meet the higher requirements of pigs fed ractopamine. Pigs fed RAC and CrY+RAC were fed CON for the first 17 d, and then the respective diets for the last 28 d on trial. Means were compared using Tukey test, excluding the CON. Dunnett test was used to compare the means of each diet containing additives to the CON. Initial BW was used as covariate. Pigs fed RAC and CrY+RAC had the highest ($P < 0.01$) final BW and ADG. Pigs fed CrY+RAC had higher ($P < 0.01$) G:F than pigs within the other groups, except for those fed RAC. Pigs fed CrY+RAC and RAC had similar G:F, both higher ($P < 0.01$) than pigs fed CON. Feed intake was similar ($P = 0.89$) for all diets. Pigs fed CrY+RAC had higher LM area ($P < 0.05$) and carcass yield ($P < 0.01$) than pigs fed CON, CrY, CLA, and CrY+CLA. Loin muscle area and carcass yield of pigs fed RAC were not statistically different from pigs fed the others diets tested. Back fat depth (BF) was lower ($P = 0.22$) in pigs fed CLA, RAC and CrY+RAC compared to pigs fed CON. Additives did not significantly affect ($P > 0.05$) pork quality, except for color: lower L^* values were found on pigs fed CrY+CLA compared to pigs fed CON ($P < 0.05$),

and RAC ($P < 0.01$). Pigs fed RAC had lower ($P < 0.05$) *a** values compared to pigs fed CON. Serum urea nitrogen concentration (SUN) was lower ($P = 0.03$) in pigs fed CrY+RAC than in pigs fed CON and RAC. In summary, it was demonstrated that, when combined, CrY and RAC increase LM area and carcass yield, and reduce SUN, suggesting a synergism that might be related to the ability of chromium to improve nutrient utilization by muscle cells in RAC-fed pigs. Additionally, the additives have no major effects on pork quality.

Key words: chromium yeast, conjugated linoleic acid, performance, pork quality, ractopamine, swine.

INTRODUCTION

Increasing swine harvest weights allows the dilution of production and processing costs (Wood and Whittemore, 2006). However, as pigs became heavier, feed efficiency is decreased due to a greater body fat accretion, worsening performance and carcass grading. Thus, raising pigs of high lean-genotypes and providing feed additives that further stimulate lean growth and minimize fat deposition are strategies to overcome this issue. Ractopamine, CLA and chromium are among the feed additives used for this purpose.

Ractopamine improves growth, feed efficiency, carcass yield, and LM area (Apple et al. 2007). The effects are mediate through the activation of β-adrenergic receptors on cell membranes, which modulates enzymatic activity and gene expression (Parr et al., 2016 "*in press*"). Ractopamine enhances protein synthesis (Bergen et al. 1989; Adeola et al. 1992) and increases the proportion of glycolytic fibers in skeletal muscle, thus favoring muscular hypertrophy (Gunawan et al., 2007). Conjugated linoleic acid modifies lipid metabolism (Park et al., 1999), and its supplementation can enhance growth and feed efficiency, reduce BF, and increase intramuscular fat content (IMF), consequently improving carcass traits and pork quality (Dugan et al. 2004). Chromium potentiates insulin action, increases glucose uptake in muscle (Evans and Bowman, 1992), and can improve pig performance and carcass leanness (NRC, 2012). Also, due to its effects on nutrient utilization, chromium could potentially intensify the effects of CLA and ractopamine supplementation. In this context, it was hypothesized that chromium yeast, alone or combined to CLA or ractopamine, could improve swine performance and carcass

traits. As increasing lean meat production can affect pork quality (Dunshea et al., 2005), the present work aimed at evaluating the effects of supplementing diets with these additives and their combination on performance, carcass traits, and pork quality of finishing pigs.

MATERIALS AND METHODS

Animals and housing

The experimental protocol has followed the ethical principles in animal research (CONCEA, 2016) and was approved by the Ethical Committee on Animal Use of Universidade Federal de Viçosa (UFV) [protocol no. 39/2014].

Ninety-six crossbred barrows (AGPIC 415 x Camborough (Agroceres PIC, Patos de Minas, MG, Brazil)) weighing 70.21 ± 1.98 kg were randomly alloted to 2.30 X 2.10 m concrete-floored pens at the swine research facility of UFV, Viçosa, Brazil. Each pen housed 2 pigs (2.42 m^2/pig) and had a dry feeder and a nipple drinker. Pigs had free access to feed and water throughout the 45 –d feeding trial. Temperature and humidity inside the barn were recorded every 3 h using a data logger.

Experimental design and diets

Pens were randomly assigned to 1 of 6 dietary treatments (Table 1). A pen with 2 pigs was considered as the experimental unit. There were 8 replicates per treatment. A diet formulated to meet the nutritional requirements of 70- to 100-kg barrows of high lean genotype gaining 1.13 kg/day (Rostagno et al., 2011) was used as the control (CON). The other 5 diets were based on the CON, and supplemented as follows: 400 ppb Cr yeast (CrY); 0.5% CLA (CLA); 400 ppb CrY and 0.5% CLA (CrY+CLA); 20 ppm RAC (RAC); 400 ppb CrY and 20 ppm RAC (CrY+RAC). The additives were added to the respective diets as follows: 0.4 kg/t of a CrY product (Alltech Inc, Nicholasville, KY); 5 kg/t of a commercial CLA preparation (BASF, Ludwigshafen, RP, Germany); and 1 kg/t of a commercial product containing 20g/kg RAC (Hertape Saúde Animal, Juatuba, MG, Brazil). Standardized ileal digestible lysine levels in the diets containing RAC were increased by 20% to account for the higher requirements reported in RAC-fed pigs, and crystalline amino acids (Met, Thr, Trp, Val) were added to reach their recommended ratio to Lys (Rostagno, 2011;

Table 1 and Table 2). Pigs assigned to RAC and CrY+RAC diets were fed the CON diet for the first 17 d on trial, and then fed the respective diets for 28 d.

Performance, blood analysis and carcass traits

Throughout the trial, feed was weighted prior to feeding and feed wastage was collected and weighted daily to determine ADFI. At the end of the experimental period, pigs were individually weighted (no fasting) to determine final BW, ADG and G:F. After that, pigs were fasted for 12 h, then allowed to consume feed *ad libitum* for 1 h, and then submitted to a 6 h-fasting period, with free access to water. Next, 1 pig per pen, with the BW closest to 115 kg, was restrained using a hog snore, and blood sample collection was performed. Ten milliliters blood samples were collected from the orbital sinus into glass tubes, using 16-gauge needles, immediately placed on ice for 30 min, then centrifuged at 2,000 x *g* for 10 min at 22°C. Sera harvested were stored at -20°C until analysis. Commercial kits (Labtest Diagnóstica S. A., Lagoa Santa, MG, Brazil) were used to analyze serum parameters. Serum glucose (GLU) was determined by glucose oxidation method (Blaedel and Uhl, 1975). Enzymatic methods were used to determine cholesterol (CHO) (Allain et al. 1974), triglycerides (TG) (Bucolo and David, 1973), and SUN (Hallet and Cook, 1971). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to Bachorik and Ross (1995).

Following blood collection, pigs were fed *ad libitum* for 6 h and then submitted to 12 h pre-slaughter fasting. After fasting, 1 pig per pen, with the BW closest to 115 kg, was individually weighted, rendered unconscious using head-only electrical stunning (240V, 1.3A), immediately exsanguinated, dehaired, and eviscerated. Carcasses were weighted to determine carcass yield using the formula: HCW divided by pre-slaughter BW, multiplied by 100. Carcasses were split longitudinally and chilled at 5°C for 24 h. All measurements and sample collections were done on the right side of each carcass. Temperature and pH of the LM were measured immediately after slaughter, 45 min, 3 h and 24 h post slaughter using a hand-held T-bar pH meter with a penetration probe and coupled thermometer (Testo SE & Co., Lenzkirch, FR, Germany) inserted in LM, near the last lumbar vertebra.

After 24h-chilling, half carcasses were split at 10^{th} rib level, and BF over the LM (6 cm away from midline) was determined using a digital caliper. To determine LM area, the muscular surface at 10^{th} rib to 11^{th}-rib interface was covered with a

polyethylene sheet, and traced using a fine point permanent marker. Sheets were scanned and colored digitally. Colored areas within the contour were measured using an image analysis software (ImageJ version 1.49 t, National Institutes of Health, Bethesda, MD).

Pork quality

A LM sample of approximately 30 cm was removed from between the 10th rib and the first lumbar vertebrae of each carcass. The muscle segment was vacuum packed, stored at -20°C for 24h, and then sequentially sectioned into five 2.54-cm chops using a table meat-saw. Chops were individually vacuum packed, identified accordingly to the animal and muscle position from where they originated from, and stored at -20°C until further analysis (Bridi and Silva, 2006).

For water losses determination, frozen chops were removed from packs, weighted, and allowed to thaw at 4°C for 12 h. After thawing, chops were gently wiped using paper towel, weighted, placed on a perforated grill, and broiled in electric oven (Layr, Luxo Inox, Jundiaí, SP, Brazil) preheated to 170°C. Temperature was monitored using a thermocouple inserted into the geometric center of the chops (Omega engineering, Stamford, CT) attached to a digital monitor. Chops were turned over when internal temperature reached 35°C, and removed from the oven when internal temperature reached 71°C. Samples were chilled at 4°C for 12 h, then re-weighted. Thaw and cook water losses were expressed as a percentage of the chop weight prior to each procedure (Bridi and Silva, 2006).

After weighing, cooked chops were used to Warner-Bratzler shear force (WBSF) determination as proposed by AMSA (2016) with minor modifications. From each sample, 6 round cores measuring 1.27 cm in diameter were removed parallel to the longitudinal orientation of the muscle fibers, using a sharp stainless steel coring device. Care was taken to avoid sampling at areas containing visible fat and connective tissue. These round cores were sheared once through the center, perpendicularly to the longitudinal orientation of the muscle fibers, using a V-notch blade with 1.016 mm thickness and 60° angle at fixed speed of 20 cm/min, coupled to a Warner-Bratzler Shear machine (G-R Electrical Manufacturing Company, Manhattan, KS). The WBSF was determined by the average of six measures and expressed in kgf.

For color evaluation, frozen chop samples were thawed at 4°C for 12 h, removed from vacuum-packs and exposed to oxygen for 30 min to allow myoglobin and oxygen to react. After that, color was determined using a handheld spectrophotometer (Hunter MiniScan EZ, 4500L; Hunter Associates Laboratory, Inc., Reston, VA), calibrated against a white and a black tile prior to data collection. The mean *L** (lightness), *a** (redness), and *b** (yellowness) values of each chop were determined as the average from three readings on three different points of chop surface, using illuminant D65, a 31.8 mm port size and a 10° standard observer (Brewer et al., 2001; Karamucki et al., 2006).

To determine IMF, chops were dissected to remove the exterior fat and connective tissue, then grinded using an electric meat grinder. A 100g-sample of each chop was evaluated for IMF using near infrared spectrophotometry (FoodScan, FOSS NIRsystems Inc., Laurel, MD) (AOAC, Official method 2007.04).

Data analysis

Data were analyzed in a randomized design, considering the initial BW as covariate, using the generalized linear models procedure (GLM proc) of SAS 9.4 (SAS Inst., Inc., Cary, NC). Means were compared using Tukey test, excluding the CON. The Dunnett test was used to compare the means of each diet containing the additives (CrY, CLA, CrY+CLA, RAC, CrY+RAC) to the CON. A pen was considered as the experimental unit for analysis of performance data (final BW, ADG, ADFI and G:F). One pig per pen was considered as the experimental unit for analysis of carcass traits, pork quality and serum parameters. For all statistical procedures, probability values lower than 0.05 were considered significant and probability values lower than 0.10 were considered as a trend.

RESULTS

Throughout the experiment, average temperature inside the barn was 22.73 ± 3.02°C. Maximum and minimum temperatures registered were 29.3°C and 15.3°C, respectively. Average humidity was 70.01% (Figure 1). The thermoneutral zone for finishing pigs ranges from 12°C to 22.5 °C (Carr, 2006), therefore, pigs were submitted to heat stress.

Pigs fed RAC and CrY+RAC had the highest ($P < 0.01$) final BW and ADG. Pigs fed CrY, CrY+CLA and CLA had similar final BW and ADG to pigs fed CON.

No differences were observed for ADFI ($P > 0.05$). Pigs fed RAC and CrY+RAC had higher ($P < 0.01$) G:F than pigs fed CON. Gain-to-feed ratio of pigs fed CrY+RAC was higher ($P < 0.01$) than G:F of pigs fed CrY, CLA, and CrY+CLA, and similar to G:F of pigs fed RAC (Table 3).

Pigs fed CrY+RAC had higher LM area ($P < 0.05$) and carcass yield ($P < 0.01$) than pigs fed CON, CrY, CLA, and CrY+CLA diets. The results of LM area and carcass yield of pigs fed RAC did not differ ($P > 0.05$) from the results of pigs fed the other four diets containing additives, nor from the CON. Back fat depth was lower ($P = 0.22$) in pigs fed CLA, RAC and CrY+RAC compared to pigs fed CON (Table 3).

No differences were observed for carcasses pH and temperature ($P > 0.05$) (Table 4). No differences were observed for water losses ($P = 0.87$) and WBSF measurements ($P = 0.70$) (Table 5). The L^* values were lower ($P < 0.05$) in chops from pigs fed CrY+CLA compared to pigs fed CON and RAC ($P < 0.01$) and similar ($P > 0.05$) to chops from pigs fed CrY, CLA, and CrY+RAC. Chops from pigs fed RAC had lower a^* values than chops of pigs fed CON ($P < 0.05$), CLA and CrY+CLA ($P < 0.01$), and similar ($P > 0.05$) a^* values to chops from pigs fed CrY and CrY+RAC. No differences ($P > 0.05$) were observed in b^* values (Table 5). There were no differences ($P = 0.89$) on IMF content ($P = 0.89$) (Table 5).

No differences were observed on GLU ($P = 0.32$), CHO ($P = 0.67$), HDL ($P = 0.76$), LDL ($P = 0.82$) and TG ($P = 0.46$) (Table 6). Serum urea nitrogen concentration was lower ($P = 0.03$) in pigs fed CrY+RAC than in pigs fed CON and RAC.

DISCUSSION

Performance and carcass traits

The higher feed efficiency of pigs fed RAC and CrY+RAC was a result of the improvements on ADG, as feed intake has not changed. Thus, it can be inferred that ADG improvements were consequential to the enhancement of protein accretion induced by ractopamine (Bergen et al. 1989; Dunshea et al., 1993a). Considering that lean mass deposition is more energetically efficient than fat mass deposition (de Lange et al., 2001) and that protein accretion is coupled with water accretion (Dunshea et al. 1993b), the improvements on growth rates and feed efficiency on

pigs fed RAC and CrY+RAC were in accordance to what would be expected. Additionally, the higher carcass yield indicates that visceral mass composed a smaller proportion of live BW in pigs fed CrY+RAC diet. As maintenance energy requirements per kilogram of protein in visceral organs is 3.1 times higher than in skeletal muscle (Noblet et al. 1999), visceral mass can decrease the efficiency of converting nutrients into valuable pork products (De Lange et al. 2001). Consequently, an increased rate of lean mass accretion relative to visceral mass may also had contribute to the observed improvements on G:F.

When combined, 20 ppm of ractopamine and 400 ppb of chromium yeast allowed substantial increases on LM area and carcass yield. Numerically, the LM areas of CrY+RAC-fed pigs were approximately 10% and 20% higher than the LM areas of pigs fed RAC and CON, respectively. Chromium improves nutrient utilization (Kornegay et al., 1997), increases insulin sensitivity (Amoikon et al. 1995), and amplifies insulin receptor signaling (Vincent, 2001), which may have provided more substrate for the already improved protein synthesis promoted by ractopamine.

The proposed mechanism by which chromium amplifies insulin signaling is related to the activity of chromodulin (Vincent, 2001), an organic complex composed by Cr^{+3} and amino acids (Yamamoto, 1987). After insulin binding, conformational changes in the insulin receptor promotes the translocation of glucose transporter type-4 to the plasma cell membrane. Concomitantly, insulin binding promotes the transportation of chromium from blood to the cytosol. Inside the cell, chromium binds to chromodulin, and chromodulin binds to the insulin receptor, further stimulating its activity, therefore increasing insulin sensitivity and enhancing glucose uptake (Vincent, 2001). Ractopamine effects are mediate through the activation of β-adrenergic receptors on cell membranes, resulting in activation of the adenylate cyclase pathway and the production of cyclic adenosine monophosphate (cAMP). As a second messenger, cAMP will activate protein kinase A. Protein kinase A alters enzymatic activity and phosphorilates the transcription factor cAMP response element binding protein (CREB), that regulates the transcription of genes that have a cAMP response element within their regulatory regions (Mersmann, 1998). In skeletal muscle cells, CREB enhances myogenic gene expression and increases mitochondrial oxidative capacity (Altarejos and Montminy, 2011). Ractopamine also alters muscle fiber composition, increasing the proportion of

glycolytic fibers (Gunawan et al. 2007), which present higher potential for hypertrophy (Lefaucher, 2010).

Gunawan et al. (2007) reported that pigs had increased mRNA expression of glycogen synthase after 1 wk ractopamine supplementation. However, after 4 wk supplementation the mRNA expression of glycogen synthase was similar to non-supplemented pigs. Based on that, it was suggested that skeletal muscle cells try to accommodate the increased glucose flow in the beginning of the ractopamine supplementation period, but, as muscle grows, energy requirement is increased, consequently reducing the availability of substrates for glycogen synthesis. If it is assumed that the magnitude of the enhancement on protein synthesis elicited by ractopamine could be limited by energy availability, then it is possible that chromium increases the energy available for protein synthesis by improving the ability of skeletal muscle cells to uptake glucose. Therefore, chromium amplifies the magnitude of lean accretion promoted by ractopamine. This hypothesis is consistent with the observed reduction on SUN in pigs fed CrY+RAC. Decreases on blood urea nitrogen are related to increased efficiency of nitrogen utilization for protein synthesis, and reduced amino acid catabolism (Bush et al., 2002). Additionally, blood urea concentration is negatively correlated with lean gain and feed efficiency (Whang and Easter, 2000).

The finding that CLA supplementation reduces BF is consistent with previous studies (Dugan et al., 2004; Dunshea et al., 2005). The reduction on BF found on pigs fed CLA was not associated with changes in feed intake. Therefore, this reduction is likely due to the influence of CLA on lipid metabolism. Mechanisms that can explain CLA lowering BF include: inhibition of lipogenesis from preformed fatty acids by decreasing lipase lipoprotein activity; inhibition of *de novo* lipogenesis by reducing mRNA abundance of acetyl-CoA carboxylase and fatty acid synthase; enhancement of lipolysis by increasing hormone sensitive lipase activity (Ostrowska et al., 2002), and enhancement of adipocytes apoptosis (Qi et al., 2014). These mechanisms were not directly evaluated on this trial. However, if the mechanisms involved inhibition of lipogenesis through decreasing activity of lipase lipoprotein, or enhancement of lipolysis, then these effects would be expected to be reflected in increased levels of serum TG and CHO, which did not happen. Based on the absence of effects on these serum parameters, an increase in adipocytes apoptosis

or an decrease in *de novo* lipogenesis are the most likely explanations for the observed reduction on BF.

Although ractopamine is generally considered to reduce lipid accretion (NRC, 2012), it is possible that the reduction on BF in pigs fed RAC and CrY+RAC is related to a dilution effect resultant from the increase in protein accretion, rather than a direct effect on blocking lipogenesis or enhancing lipolysis (Dunshea and King, 1994; 1995). The present results endorse the dilution effect hypothesis, as no changes were observed on blood metabolites that would suggest changes in lipolysis or lipogenesis rate. However, it is also possible that the length of supplementation period might have triggered the down-regulation of the β-adrenergic receptors on fat cell surfaces (Spurlock et al. 1994), therefore changes in lipolysis or lipogenesis rate at the beginning of the supplementation period would not be present when blood samples were collected at the end of the trial.

Pork quality

One of the most important determinants of meat quality is pH, because it directly influences meat color and water holding capacity, which will, in turn, influence juiciness, tenderness, flavor, shelf life and consumers' acceptance of pork. Anaerobic glycolysis is the key metabolic pathway on the conversion of muscle to meat: as glycogen and ATP levels in muscle decline, lactic acid accumulates, thus lowering muscle pH. When glycolysis rate is accelerated before slaughter, it will also be accelerated on early *post mortem*, therefore increasing the production of lactic acid. Consequently, there is a rapid decline on muscle pH when the carcass temperature is still high. The combination of low pH and high temperature causes protein denaturation, which increases the chances of producing PSE pork (Wismer-Pedersen, 1959). The selection of pigs for maximum lean gain increases the proportion of glycolytic fibers in muscle (Rehfeldt et al. 2008). Glycolytic fibers have higher myosin ATPase activity, higher concentration of glycogen, and higher capacity to metabolize glycogen under anaerobic conditions than oxidative muscle fiber type. Therefore, higher proportions of glycolytic fibers can increase the occurrence of PSE pork (Bowker et al., 2000).

In this context, monitoring pH and temperature decline in swine muscle is of paramount importance, especially when pigs are supplemented with additives that can modulate muscle metabolism. Ractopamine supplementation increases the

expression of myosin heavy chain type IIb in pig skeletal muscle, which is characteristic of glycolytic muscle fiber (white, fast-twitch) (Depreux et al., 2002). Chromium supplementation is reported to increases the expression of glycogen synthase mRNA in muscle cell culture media (Qiao et al. 2009), which indicates an increase in the proportion of glycolytic fibers (Shen et al., 2015).

Despite the reported effects on muscle fiber type, none of the additives influenced the rate of pH decline or temperature, which is consistent with the absence of effects on water losses and WBSF. When pH 24 *post mortem* is lower than 5.5, it is expected that water holding capacity is impaired mainly due to the denaturation of myofibrillar proteins (Aaslying et al., 2003). Impairment of water holding capacity leads to higher cooking losses, which negatively affect meat juiciness and tenderness. In this study, no differences were observed in WBSF of chops from pigs fed RAC, indicating that RAC has no detrimental effect on pork quality. The WBSF results contradict previous studies that indicated that RAC supplementation potentially increases pork WBSF due to its effects on reducing activity of proteolytic enzymes (Sainz et al, 1993) and IMF (Fortin et al. 2005). Although there were no effects on pH, the additives had a small influence on pork color. Lower a^* values found on pork chops from pigs fed RAC can be attributable to an increase in the proportion of glycolytic muscle fibers, thus decreasing the amount of oxymyoglobin present (Uttaro et al., 1993). Considering the inconsistency and scarcity of reported effects of chromium (Joo et al., 2000; Bucko et al., 2013) and CLA (Wiegand et al. 2001; Wiegand et al., 2002) supplementation on pork color, a possible explanation for the decrease in L^* values observed on loins chops from pigs fed CrY+CLA remains elusive.

Longissimus muscle chops obtained from pigs fed CLA had a numerical increase of approximately 20% on IMF compared to CON. Dietary CLA supplementation consistently decreases BF and reduces IMF on pork (Dugan et al., 2004; Dunshea et al., 2005). As previously discussed, CLA regulates the activity of enzymes involved on lipid metabolism. However, the responsiveness to CLA is different on subcutaneous and intramuscular fat deposits (Zhou et al. 2007; Jiang et al., 2010). For instance, the activity of fatty acid synthase and lipoprotein lipase, which were reported to be reduced in subcutaneous fat after CLA supplementation, were not affected on LM (Jiang et al., 2010). Additionally, CLA deposition rate is higher in BF than in LM (Cordero et al. 2010; Jiang et al., 2010), leading to

differences on CLA concentration that could further explain the opposite effects (McNeel and Mersmann, 2003). This suggestion is reinforced by Jiang et al. (2010), who reported increased adipocyte proliferation when porcine preadipocytes cell culture media was enriched with 50 to up to 350 μM CLA; but decreased in adipocytes proliferation when CLA concentration was raised to 400 μM. A shift on muscle metabolism, leading towards an increase in oxidative muscle fibers, which have higher lipid content compared to glycolytic fibers (Essén-Gustvsson et al., 1994), could also explain the observed effect on IMF. In this context, CLA supplementation potentially benefits pork production, as IMF positively influences sensory qualities of pork (Daszkiewick et al., 2005), whereas subcutaneous fat must be minimize to improve carcass grading. Noteworthy, IMF on chops obtained from ractopamine-fed pigs were similar to IMF observed for pigs fed CON. The fact that ractopamine does not reduce IMF is an important indication that its supplementation does not impair pork eating quality.

CONCLUSION

Ractopamine improves growth rate and feed efficiency, and decreases BF of finishing pigs. When combined, 20 ppm ractopamine and 400 ppb chromium yeast elicit significant increases on LM area and carcass yield, suggesting the existence of a synergistic effect that could be related to the ability of chromium yeast to improve nutrient utilization in pigs fed ractopamine. Animal performance is not affected by either CrY or CLA supplementation. Conjugated linoleic acid supplementation reduces BF and may improve IMF. Supplementing finishing pigs' diets with Cr, CLA and RAC exert no major effects on pork quality.

ACKNOWLEDGMENTS

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Ciência e Tecnologia e Tecnologia de Ciência Animal (INCT-CA) for financial support.

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Table 1. Composition (% as fed) of experimental diets

Ingredients, %	Diets					
	CON	CrY	CLA	CrY+CLA	RAC	CrY+RAC
Corn	76.05	76.05	76.05	76.05	76.05	76.05
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean oil	0.55	0.55	0.55	0.55	0.35	0.35
Dicalcium phosphate	0.87	0.87	0.87	0.87	0.87	0.87
Calcium carbonate	0.61	0.61	0.61	0.61	0.61	0.61
Inert clay filler	0.70	0.66	0.20	0.16	0.30	0.26
Salt	0.36	0.36	0.36	0.36	0.36	0.36
L-lysine HCl 99%	0.24	0.24	0.24	0.24	0.45	0.45
DL-methionine 99%	0.05	0.05	0.05	0.05	0.15	0.15
L-threonine 98.5%	0.05	0.05	0.05	0.05	0.16	0.16
L-tryptophan	-	-	-	-	0.03	0.03
L-valine	-	-	-	-	0.05	0.05
Vitamin premix[1]	0.28	0.28	0.28	0.28	0.28	0.28
Mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20
Tylan G250[3]	0.01	0.01	0.01	0.01	0.01	0.01
Pulmolin[4]	0.02	0.02	0.02	0.02	0.02	0.02
CLA[5]	-	-	0.50	0.50	-	-
Chromium yeast[6]	-	0.04	-	0.04	-	0.04
Ractopamine[7]	-	-	-	-	0.10	0.10
Antioxidant[8]	0.01	0.01	0.01	0.01	0.01	0.01

[1] Provided per kilogram of the complete diet: vitamin A, 5,880 IU; vitamin D_3, 980 IU; vitamin E, 14 IU; vitamin K_3, 2.38 mg; vitamin B6, 0.7 mg; vitamin B1, 3.7 mg; vitamin B1, 0.7 mg; vitamin B12, 0.7 mcg; selenium 0.21 mg; calcium pantothenate, 11.2 mg; biotin, 0.04 mg; niacin, 2.30 mg; folic acid, 0.35 mg.
[2] Provided per kilogram of the complete diet: Fe as ferrous sulfate, 40 mg; Cu as copper sulfate, 112 mg; I as calcium iodate, 0.8 mg; Zn as zinc oxide, 64 mg, and Mn as manganese sulfate, 28 mg.
[3] 250 g tylosin/kg (Tylan G250, Elanco, São Paulo, SP, Brazil).
[4] 200 g tiamulin/kg (Pulmolin, Farmabase, Jaguariúna, SP, Brazil).
[5] Minimum 56% of CLA methyl esters, 50:50 *cis-9,trans11*:*t10c12* (BASF).
[6] Co-factor III (Alltech Inc., Nicholasville, KY).
[7] 20 g ractopamine/kg (Ractop, Hertape Saúde Animal)
[8] 65g/kg butylated hydroxytoluene; 15g/kg etoxiquin and 7g/kg butylated hydroxyanisole (Banox 100, Alltech Inc., Nicholasville, KY).

Table 2. Calculated nutritional composition of experimental diets[1]

Item[2]	Diets[3]					
	CON	CrY	CLA	CrY+CLA	RAC	CrY+RAC
ME, kcal/kg	3230	3230	3230	3230	3230	3230
Crude protein, %	15.33	15.33	15.33	15.33	15.33	15.33
SID Lys, %	0.83	0.83	0.83	0.83	1.00	1.00
SID Met + Cys, %	0.50	0.50	0.50	0.50	0.60	0.60
SID Thr, %	0.56	0.56	0.56	0.56	0.67	0.67
SID Trp, %	0.15	0.15	0.15	0.15	0.18	0.18
SID Val, %	0.64	0.64	0.64	0.64	0.69	0.69
Na, %	0.16	0.16	0.16	0.16	0.16	0.16
Ca, %	0.51	0.51	0.51	0.51	0.51	0.51
Available P, %	0.25	0.25	0.25	0.25	0.25	0.25

[1]Values calculated according to Rostagno et al. (2001).
[2] SID = standardized ileal digestible
[3] CON = control diet; CrY = 400 ppb chromium yeast; CLA = 0.5% CLA; CrY + CLA = 400 ppb chromium yeast + 0.5% CLA; RAC = 20 ppm ractopamine; CrY + RAC = 400 ppb chromium yeast + 20 ppm ractopamine

Table 3. Effects of dietary chromium, CLA, and ractopamine on performance and carcass traits of finishing pigs

Item[2]	Diets[1]						P-value	
	CON	**CrY**	**CLA**	**CrY+CLA**	**RAC**	**CrY+RAC**	Dunnett	Tukey
IBW[4], kg	70.35	70.22	70.00	70.38	70.14	70.17		
FBW[4], kg	120.0 ± 1.3	118.0 ± 1.3^{C}	122.4 ± 1.3^{B}	120.6 ± 1.4^{BC}	127.1 ± 1.4$^{A\Psi}$	127.9 ± 1.3$^{A\Psi}$	<0.01	<0.01
ADG, g/d	1107 ± 30	1036 ± 30^{C}	1161 ± 30^{B}	1121 ± 32^{BC}	1264 ± 32$^{A\Psi}$	1284 ± 30$^{A\Psi}$	<0.01	<0.01
ADFI, g/d	3086 ± 70	3088 ± 70	3119 ± 70	3009 ± 70	3107 ± 72	3121 ± 70	0.89	0.79
G:F	0.358 ± 0	0.344 ± 0^{D}	0.372 ± 0^{BC}	0.369 ± 0^{C}	0.395 ± 0$^{AB\Psi}$	0.415 ± 0$^{A\Psi}$	<0.01	<0.01
LMA, cm^2	50.61 ± 1.9	50.61 ± 1.9^{B}	51.11 ± 1.9^{B}	48.45 ± 2.0^{B}	55.53 ± 2.0^{AB}	60.71 ± 2.0$^{A\Psi}$	<0.01	<0.01
CY, %	81.39 ± 0.4	81.26 ± 0.4^{B}	81.24 ± 0.4^{B}	81.08 ± 0.4^{AB}	82.11 ± 0.4^{AB}	83.15 ± 0.4$^{A\Psi}$	<0.05	<0.05
BF, mm	17.31 ± 1.0	13.80 ± 1.0	12.54 ± 1.0$^{\Psi}$	14.01±1.07	13.29±1.07$^{\Psi}$	12.84±1.0$^{\Psi}$	0.022	0.79

[1] CON = control diet; CrY = 400 ppb chromium yeast; CLA = 0.5% CLA; CrY + CLA = 400 ppb chromium yeast + 0.5% CLA; RAC = 20 ppm ractopamine; CrY + RAC = 400 ppb chromium yeast + 20 ppm ractopamine

[2] IBW = initial body weight; FBW = final body weight; LMA = loin muscle area; CY = carcass yield

[3] Data presented represent the mean value and standard error of means in 16 pigs/treatment (sample size = 96)

[4] Initial body weight were used as a covariable for analysis of variance of performance and carcass traits.

[5] Data presented represent the mean value and standard error in 8 pigs/treatment (sample size = 48)

$^{\Psi}$ Mean values within a row differ from control (P<0.05) using Dunnett test.

$^{A, B, C}$ Excluding the CON, mean values within a row without a common superscript differ (P<0.05) using the Tukey test.

Table 4. Effects of dietary chromium, CLA, and ractopamine on pH and carcass temperature of finishing pigs[1,2]

Item	Diets[3]						P-value	
	CON	**CrY**	**CLA**	**CrY+CLA**	**RAC**	**CrY+RAC**	Dunnett	Tukey
pH								
0 min	6.14 ± 0.09	6.29 ± 0.09	6.23 ± 0.09	6.11 ± 0.09	6.16 ± 0.09	6.29 ± 0.09	0.643	0.564
45 min	5.77 ± 0.01	5.95 ± 0.01	5.87 ± 0.01	5.99 ± 0.01	5.97 ± 0.01	5.93 ± 0.01	0.776	0.954
3 h	5.53 ± 0.1	5.76 ± 0.1	5.66 ± 0.1	5.79 ± 0.1	5.78 ± 0.1	5.70 ± 0.1	0.573	0.839
24 h	5.36 ± 0.1	5.34 ± 0.1	5.26 ± 0.1	5.33 ± 0.1	5.39 ± 0.1	5.46 ± 0.1	0.309	0.259
Temperature								
0 min	34.8 ± 0.5	35.1 ± 0.5	35.2 ± 0.5	35.9 ± 0.5	34.4 ± 0.5	36.5 ± 0.5	0.106	0.106
45 min	29.6 ± 1.0	30.3 ± 1.0	29.7 ± 1.0	29.1 ± 1.0	29.3 ± 1.0	30.2 ± 1.0	0.955	0.955
3 h	23.3 ± 0.7	20.6 ± 0.7	22.1 ± 0.7	21.4 ± 0.7	22.0 ± 0.7	22.5 ± 0.7	0.139	0.139
24 h	8.2 ± 0.7	7.8 ± 0.7	8.0 ± 0.7	7.8 ± 0.7	8.0 ± 0.7	8.0 ± 0.7	0.966	0.966

[1] Data presented represent the mean value and standard error of means in 8 pigs/treatment (sample size = 48)
[2] Initial body weight were used as a covariable for analysis of variance of pH and carcass temperature
[3] CON = control diet; CrY = 400 ppb chromium yeast; CLA = 0.5% CLA; CrY + CLA = 400 ppb chromium yeast + 0.5% CLA; RAC = 20 ppm ractopamine; CrY + RAC = 400 ppb chromium yeast + 20 ppm ractopamine.

Table 5. Effects of dietary chromium, CLA, and ractopamine on pork quality parameters evaluated on LM of finishing pigs[1,2]

Item[4]	Diets[3]						P-value	
	CON	**CrY**	**CLA**	**CrY + CLA**	**RAC**	**CrY + RAC**	Dunnett	Tukey
TL, %	9.3 ± 0.9	9.6± 0.9	9.2 ± 1.0	9.4± 0.9	9.9 ± 1.0	8.2± 0.9	0.874	0.679
CL, %	27.0 ± 1.2	29.0 ± 1.2	28.1 ± 1.2	28.5 ± 1.2	27.9 ± 1.2	27.4 ± 1.2	0.872	0.867
SL, %	33.9 ± 1.2	35.8 ± 1.2	34.2 ± 1.2	35.2 ± 1.2	35.3 ± 1.2	33.4 ± 1.2	0.716	0.611
WBSF, kgf	4.2 ± 0.3	4.8 ± 0.3	4.6 ± 0.3	4.8 ± 0.3	4.4 ± 0.3	4.8 ± 0.4	0.703	0.894
*L**	52.5 ± 0.7	51.7 ± 0.7 ^{AB}	51.8 ±0.7^{AB}	50.3 ± 0.7$^{B\psi}$	54.1 ± 0.7^{A}	53.2 ± 0.7^{AB}	<0.05	<0.01
*a**	8.7 ± 0.5	8.5 ± 0.5^{AB}	9.2 ± 0.5^{A}	8.8 ± 0.5^{A}	6.9 ± 0.5$^{B\psi}$	7.8 ± 0.5^{AB}	<0.05	<0.01
*b**	15.4 ± 0.3	14.9 ± 0.3	15.7 ± 0.3	14.8 ± 0.3	14.6 ± 0.3	7.5 ± 0.3	0.203	0.203
IMF, %	1.33 ± 0.2	1.16 ± 0.2	1.60 ± 0.2	1.36 ± 0.2	1.37 ± 0.2	1.30 ± 0.2	0.860	0.878

[1] Data represent the mean value and standard error of means in 8 pigs/treatment (sample size = 48)
[2] Initial body weight were used as a covariable for analysis of variance of pork quality parameters
[3] CON = control diet; CrY = 400 ppb chromium yeast; CLA = 0.5% CLA; CrY + CLA = 400 ppb chromium yeast + 0.5% CLA; RAC = 20 ppm ractopamine; CrY + RAC = 400 ppb chromium yeast + 20 ppm ractopamine
[4] TL = Thaw water losses; CL = cooking water losses; SL = sum of water losses; WBSF=Warner Bratzler shear force; IMF = intramuscular fat content
$^{\Psi}$ Mean values within a row differ from control (P<0.05) using Dunnet test.
$^{A, B, C}$ Excluding the CON, mean values within a row without a common superscript differ (P<0.05) using the Tukey test.

Table 6. Effects of dietary chromium, CLA and ractopamine on serum constituents in finishing pigs[1,2]

Item[4]	Diets[3]						P-value	
	CON	**CrY**	**CLA**	**CrY + CLA**	**RAC**	**CrY + RAC**	Dunnett	Tukey
GLU, mg/dl	68.0 ± 5.6	65.6 ± 3.2	70.0 ± 3.2	76.0 ± 3.2	72.3 ± 3.2	68.8 ± 3.21	0.32	0.23
CHO, mg/dl	78.2 ± 8.1	73.0 ± 5.3	76.4 ± 5.0	85.1 ± 5.0	80.0 ± 5.3	79.6 ± 5.0	0.67	0.52
HDL, mg/dl	36.0 ± 4.2	38.9 ± 2.4	39.0 ± 2.4	41.8 ± 2.4	42.0 ± 2.4	40.7 ± 2.4	0.76	0.82
LDL, mg/dl	42.3 ± 6.1	36.0 ± 2.9	39.3 ± 3.3	37.0 ± 3.0	38.9 ± 3.3	35.2 ± 3.0	0.82	0.84
TG, mg/dl	33.8 ± 5.6	24.5 ± 3.2	29.0 ± 3.2	32.8 ± 3.2	31.3 ± 3.2	27.7 ± 3.2	0.46	0.43
SUN, mg/dl	32.2 ± 1.38	31.1 ± 1.4^{AB}	31.4 ± 1.6^{AB}	31.0 ± 1.4^{AB}	32.2 ± 1.4^{A}	25.7 ± 1.5$^{B\psi}$	0.03	0.03

[1] Data presented represent the mean value and standard error of means in 8 pigs/treatment (sample size = 48)
[2] Initial body weight were used as a covariable for analysis of variance of serum constituents
[3] CON = control diet; CrY = 400 ppb chromium yeast; CLA = 0.5% CLA; CrY + CLA = 400 ppb chromium yeast + 0.5% CLA; RAC = 20 ppm ractopamine; CrY + RAC = 400 ppb chromium yeast + 20 ppm ractopamine
[4] GLU = glucose; CHO = cholesterol; HDL = high-density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; SUN = serum urea nitrogen
$^{\Psi}$ Mean values within a row differ from control ($P<0.05$) using Dunnet test.
$^{A, B, C}$ Excluding the CON, mean values within a row without a common superscript differ ($P<0.05$) using the Tukey test.

Figure 1. Environmental temperature (T) and humidity (H) throughout the trial